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Effects of invasive fish and temperature on the foraging success of Southern Iberian chub

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“But the reason I call myself by my childhood name is to remind myself that a scientist must also be absolutely like a child. If he sees a thing, he must say that he sees it, whether it was what he thought he was going to see or not. See first, think later, then test. But always see first. Otherwise you will only see what you were expecting.”

— Douglas Adams (1984), “*So Long, and Thanks for All the Fish*”.

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ABSTRACT

The number of species introduced worldwide has more than doubled over the last couple of decades. Freshwater ecosystems are one of the most diverse and threatened ecosystems in the world, with over 29% freshwater species threatened with extinction and 20% of all assessed freshwater fish listed as threatened by non-native species. Efforts to minimize the effects of invaders and to preserve declining native species are complicated by the complexity of the combined influences of multiple biotic interactions and abiotic factors, such as temperature, that could significantly drive the impact of invasive species on recipient ecosystems.

Climate change is expected to cause a global warming of about 1-5ºC relative to 1986–2005 over the 21st century, that may strongly mediate biological invasions in freshwater ecosystems. Temperature-specific competition may particularly important when studying the impacts of invasive species on freshwater biota, in future altered climates. The Iberian Peninsula harbours a rich and highly endemic freshwater fish fauna, is among the most heavily invaded systems in the world, and is also potentially susceptible to significant climatic changes. In this context, it will be important to understand to what extent increased temperatures will affect interactions between invasive and native fish in the region, and whether that could result in changes in competitive dominance between species.

The objective of this dissertation was to experimentally assess the effects of two ecologically damaging invasive fishes on the foraging success of a typical Iberian fish and how these can be mediated by temperature. Model species were the native Southern Iberian chub *Squalius pyrenaicus*, and the invasive pumpkinseed sunfish *Lepomis gibbosus* and chameleon cichlid *Australoheros facetus*, which have variable temperature tolerances that could influence the outcomes of competition for food.

Experimental trials were designed to test for the effect of temperature on the foraging success of each species when on its own and on foraging success of chub when in the presence of another species, either another chub or each of the invasive species. The experiments included three single species groups, one for each species, and three paired species groups, of two chubs and of chub with each of the invasive species. Each species group was tested at 19, 24 and 29ºC, to represent, at least partially, the range of conditions in future climates in Iberian fresh waters, resulting in a total of 18 trials. Each trial consisted of 10 deliveries of *Chironomidae* larvae at two-minute intervals. Foraging success by each fish was derived from the number of prey captured and the mean time to capture prey. Variation in these metrics was tested among temperature treatments for each species group and for the same temperature among species groups.

Temperature did not significantly affect the number of, or the speed with which, the chub captured prey, both when alone and when competing with a conspecific. However, chub tended to underperform, in terms of speed, at 24ºC and there was a slight trend indicating the increase in prey capture with the rise in temperature, when foraging alone. Chub only captured, on average across temperatures, about half as many prey as either invasive in single trials. In single trials, pumpkinseed showed a slight advantage over chameleon cichlid and more considerably so in relation to chub, at 19ºC, whereas at 24ºC chameleon cichlid captured more prey and did so faster than chub and pumpkinseed. At 29ºC, pumpkinseed and chameleon cichlid were at their fastest and all species captured more prey than at the other temperatures. When in paired trials, both invasive species outperformed the chub and captured more preys at all temperatures and chub took less time to capture preys when in the presence of the invasive species, than when competing with another chub. Whilst temperature did not affect the foraging success of chub across species groups, for each temperature treatment, foraging success varied depending on the species competing with the chub. At 19 and 24ºC, chub captured significantly less

prey when competing with chameleon cichlid, than when with a conspecific, whereas at 29ºC this occurred when with either invasive species.

Our results indicate that both pumpkinseed and the chameleon cichlid have negative effects on the foraging success of the native chub and that these effects are heightened as temperature rises when with chameleon cichlid, but are less accentuated when with pumpkinseed. This suggests that competition between native and invasive fish in Iberian fresh waters may become more likely at the higher temperatures expected under future altered climates, with advantages for invaders. Clarifying further the multiple influences on fish foraging success will be critical to better predict the evolution of species interactions as the climate changes, and enhance our ability to manage and preserve Iberian freshwater ecosystems.

Key-words: Cyprinidae; foraging success; invasive fish; climate change; Iberian Peninsula

RESUMO

O número de espécies introduzidas mundialmente mais do que duplicou nas últimas duas décadas, e a disseminação de espécies não-nativas constitui uma ameaça para a conservação da biodiversidade e agora é considerada uma grave questão ambiental, de interesse público. Os ecossistemas dulçaquícolas são um dos ecossistemas mais diversos e ameaçados no mundo, com mais de 29% de espécies dulçaquícolas ameaçadas de extinção e 20% de todos os peixes dulçaquícolas avaliados como ameaçados por espécies não-nativas. As espécies invasoras podem interagir de múltiplas formas negativas com as espécies nativas, e podem causar declínios rápidos e a extinção de algumas. Os esforços para minimizar os efeitos das espécies invasoras e para melhorar a nossa capacidade de preservar as espécies nativas em declínio são dificultados pela complexidade das influências combinadas de múltiplas interações bióticas e das suas relações com fatores abióticos, como a temperatura, que podem influenciar significativamente o impacto das espécies invasoras nos ecossistemas recetores.

Prevê-se que as mudanças climáticas provoquem um aquecimento global de cerca de 1-5ºC em relação a 1986-2005, ao longo do século XXI, e que isto medie fortemente as invasões biológicas nos ecossistemas dulçaquícolas. A competição específica de temperatura pode ser particularmente importante ao estudar os impactos de espécies invasoras sobre as espécies nativas de ecossistemas de água doce, em climas alterados futuros. A Península Ibérica alberga uma fauna de peixes de água doce rica em espécies endémicas, uma grande proporção das quais é atualmente de elevada preocupação de conservação e se encontra muito ameaçada pela degradação e fragmentação do habitat e por invasões biológicas. Os peixes de água doce ibéricos são muito sensíveis aos impactos das espécies invasoras, sendo este impacto considerado como a principal causa de declínio de peixes nativos. De fato, as águas doces ibéricas estão entre os sistemas mais invadidos do mundo, com cada vez mais espécies novas de peixes sendo introduzidas, e espécies previamente estabelecidas expandindo as suas distribuições. A Península Ibérica é potencialmente suscetível a mudanças climáticas significativas, com um aquecimento médio previsto de cerca de 5ºC, em relação a 1986-2005, e potencialmente superior a 7°C, resultando na seca substancial e aquecimento da região, e aumento da variabilidade inter-anual na ocorrência de eventos extremos de calor e de seca. Neste contexto, será importante compreender até que ponto o aumento da temperatura afetará as interações entre peixes invasores e nativos e se isso poderá resultar em mudanças nas relações de competição entre espécies, de modo a prever possíveis tendências futuras em termos de invasões biológicas e perda de biodiversidade.

O objetivo desta dissertação foi avaliar experimentalmente os efeitos da presença de duas espécies de peixes invasoras ecologicamente prejudiciais no sucesso alimentar de um peixe ibérico típico, e a forma como esses efeitos podem ser mediados pela temperatura. As espécies utilizadas neste estudo foram o escalo do Sul *Squalius py*renaicus, e a perca-sol *Lepomis gibbosus* e o chanchito *Australoheros facetus*, que possuem tolerâncias de temperatura potencialmente variáveis que poderiam influenciar os resultados da competição por alimento.

Os indivíduos utilizados nas experiências foram amostrados em ribeiras da Bacia do Sado, por pesca elétrica, entre Janeiro de 2016 e Maio de 2017. Os ensaios experimentais foram desenhados para testar o efeito da temperatura sobre o sucesso alimentar de cada espécie individual, e sobre o sucesso alimentar do escalo quando na presença de outra espécie, ou outro escalo ou uma das espécies invasoras. As experiências incluíram três grupos de uma espécie única, um para cada espécie, e três grupos de pares de espécies, de dois escalos e de escalo com cada uma das espécies invasoras. Cada grupo de espécies foi testado a 19, 24 e 29ºC, temperaturas que representam, pelo menos parcialmente, a variedade de condições que podem vir a ocorrer futuramente na região do Sado, já que a temperatura máxima da água

registada durante as amostragens realizadas foi de 24ºC e o aumento esperado da temperatura do ar para a região é de cerca de 5ºC (i.e. 24±5ºC). No total este desenho resultou num total de 18 tipos de ensaios, os quais foram replicados entre 6 e 9 vezes.

Cada experiência incluiu de 10 entregas de uma larva de *Chironomidae*, com intervalos de pelo menos dois minutos. O sucesso alimentar de cada indivíduo foi quantificado a partir do número de presas capturadas e do tempo médio de captura das presas. A variação nestas métricas foi testada usando o teste não-paramétrico de Kruskal-Wallis, e o teste a posteriori de comparação múltipla de Dunn. Para os ensaios de uma única espécie, foram feitas comparações entre tratamentos de temperatura para cada espécie e para a mesma temperatura entre espécies. Para os ensaios de pares de espécies, comparamos os mesmos pares de espécies entre temperaturas e entre pares de espécies, à mesma temperatura.

Em ensaios de uma única espécie, o número de presas capturadas pelo escalo não foi significativamente diferente entre as temperaturas. Por outro lado, a perca-sol capturou significativamente menos presas a 24ºC do que a 29ºC. Da mesma forma, o chanchito mostrou uma ligeira tendência para capturar menos presas a 19ºC, do que nas duas temperaturas mais elevadas. A 19ºC, não foram encontradas diferenças significativas no número de presas capturadas entre espécies . No entanto, a 24ºC, o chanchito capturou significativamente mais presas do que o escalo e a perca-sol, e a 29ºC, o escalo capturou significativamente menos presas do que o chanchito. O tempo médio para capturar presas não foi avaliado para o escalo a 19ºC devido a existirem poucas observações (N<5). O escalo não mostrou diferenças significativas no tempo para capturar presas entre 24 e 29ºC. Do mesmo modo, não foi encontrada nenhuma variação para a perca-sol entre os três tratamentos de temperatura, embora tenha ocorrido uma ligeira tendência para capturar presas mais lentamente a 24ºC. Por outro lado, o chanchito capturou presas de forma significativamente mais rápida nos dois tratamentos de alta temperatura, do que a 19ºC. A 24ºC, o chanchito capturou presas cerca de 30 vezes mais rápido do que o escalo. A 29ºC, ambas as espécies invasoras capturaram as presas 10 vezes mais rápido do que o escalo. Em ensaios de pares de espécies, o número de presas capturadas pelo escalo em diferentes temperaturas, não foi significativamente diferente. A 19ºC, o escalo capturou significativamente mais presas quando na presença de outro escalo do que do chanchito. A 24ºC, o escalo capturou significativamente mais presas, tanto quando com outro escalo como com a perca-sol, do que com o chanchito. A 29ºC, o escalo capturou significativamente mais presas quando com outro escalo do que quando com qualquer das espécies invasoras. Não houve diferenças significativas no tempo que o escalo demorou a capturar presas entre os 19, 24 e os 29ºC, quando na presença de outro escalo. Quando com a perca-sol, o tempo necessário para capturar presas foi semelhante aos 19 e 24ºC. Ao competir com o chanchito a 19 e 29ºC, o tempo que escalo levou a capturar presas também foi semelhante. A 19ºC, o escalo capturou presas mais rapidamente quando com a perca-sol do que com um conspecífico, mas três vezes mais lento quando com o chanchito. A 24ºC, escalo foi significativamente mais rápido quando com perca-sol, do que com outro escalo. A 29ºC, o escalo foi mais rápido quando competia com perca-sol e mais lento quando com chanchito, em comparação com quando com outro escalo.

Estes resultados indicam que a perca-sol e o chanchito têm um efeito negativo sobre o sucesso alimentar do escalo nativo, e que esse efeito é acentuado pelo aumento de temperatura quando na presença de chanchito, enquanto que as interações entre escalo e perca-sol são menos acentuadas e mais variadas a todas as temperaturas. Em geral, os resultados deste estudo sugerem que a competição entre peixes nativos e invasores em ecossistemas dulçaquícolas ibéricos se pode tornar mais provável sob as temperaturas mais altas esperadas em futuros climas alterados, com vantagens para os invasores. Isso poderá contribuir para um aumento do sucesso de introdução e estabelecimento de espécies invasoras, possivelmente levando ao aumento de deslocamento de espécies nativas, mudanças na dieta e perda de biodiversidade. Para compreender melhor as interações complexas que poderão ameaçar a fauna

dulçaquícola Ibérica sob climas futuros, será importante, por exemplo, realizar testes de sucesso alimentar sob uma maior variedade de temperaturas, avaliar o efeito de tamanho e de estágios de ontogenia dos peixes, e analisar a influência de outras variáveis, como o fluxo e a turbidez, que podem também vir a variar no futuro. Clarificar as múltiplas influências que afetam o sucesso alimentar das espécies dulçaquícolas será fundamental para prever melhor a evolução das interações entre espécies à medida que o clima muda e aprimorar a nossa capacidade de gerir e preservar os ecossistemas de água doce ibéricos.

Palavras-chave: Cyprinidae; sucesso alimentar; peixes invasores; alterações climáticas; Península Ibérica.

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1. INTRODUCTION

1.1 BIOLOGICAL INVASIONS

As the number of species introduced worldwide have more than doubled over the last couple of decades, in association with growth in global trade and human mobility (Williamson & Fitter 1996, Gozlan 2008), the introduction of non-native species into new environments and their spread over natural areas poses important threats for the conservation of biodiversity (Clavero & García-Berthou 2005). Biological invasions are now considered a major environmental issue of public concern, and have been identified as the main cause of worldwide extinction of birds (65 out of 129 spp.) (BirdLife International 2000), fish (11 out of 23 spp.) (Harrison & Stiassny 2004) and mammals (12 out of 25 spp.) (McPhee & Flemming 2004).

Although freshwater ecosystems cover less than 1% of the planet, they are one of the most diverse and threatened ecosystems in the world (Strayer & Dudgeon 2010). Of the 25,007 freshwater species assessed globally in The IUCN Red List of Threatened Species™, over 29% are being threatened with extinction (IUCN 2013), with this percentage expected to increase (IUCN 2013). Freshwater ecosystems harbour about 40 % of the world's fishes (Poff et al 2012). In an update of the IUCN Red List of Threatened Species™ in 2009, of the 3,120 freshwater fish species assessed, 3% were extinct and 37% classified as threatened ("Critically Endangered", "Endangered" or "Vulnerable"), with 20% of all assessed freshwater fish listed as threatened by non-native species (Olden et al 2007). The current global freshwater biodiversity crisis stems from many types of human activity, including the introduction and spread of harmful non-native species (Strayer and Dudgeon 2010), with as many as 35 freshwater fish species introduced to Europe from North America, before 1990, of which 12 successful established themselves and spread (Jeschke & Strayer 2007). Worldwide, the number of fish species introduced from known sources, since 1950, reaches 624 species of which 91% is explained by aquaculture (51%), ornamental fish (21%), angling or sport (12%) or fisheries (7%) (Gozlan 2008).

Invasive species may interact in multiple negative ways with native biota and can cause rapid population declines and the extinction of native species, mainly through predation and competitive behaviour strategies, that were absent during the evolution of isolated native populations (Mills et al 2004, Clavero & García-Berthou 2005, Caiola & Sostoa 2005). Efforts to minimize the effects of invasive species on the native fish fauna and to improve our ability to preserve declining native species, are complicated by the complexity of the combined influences of multiple biotic interactions, such as predation, aggression/behavioural interference and resource competition (Baxter et al 2004; Mills et al 2004; Ficke et al 2007; Leunda 2010), and their relation to abiotic factors, such as temperature, that could significantly shape the impact of invasive species on recipient ecosystems (Ficke et al 2007).

1.2 CLIMATE CHANGE

Climate change, the widespread and prolonged alteration in weather patterns across the globe we are currently experiencing, caused by the increase of anthropogenic greenhouse gas emissions since the industrial revolution, has led to increased temperature trends across the planet since the mid-20th century (NASA). Generally described as global warming, these trends have involved a rise of between 0.2 and 0.6ºC in global mean temperatures in the last 50 years. Sixteen of the 17 warmest years in the 136-year worldwide records all have occurred since 2001, and surface temperature is forecasted to rise about 1-5ºC relative to 1986–2005, over the 21st century under all assessed emission scenarios, with more

intense and frequent heat waves and extreme precipitation events in many regions (Santos & Miranda 2006, IPCC 2014, NASA) (Figure 1.1).

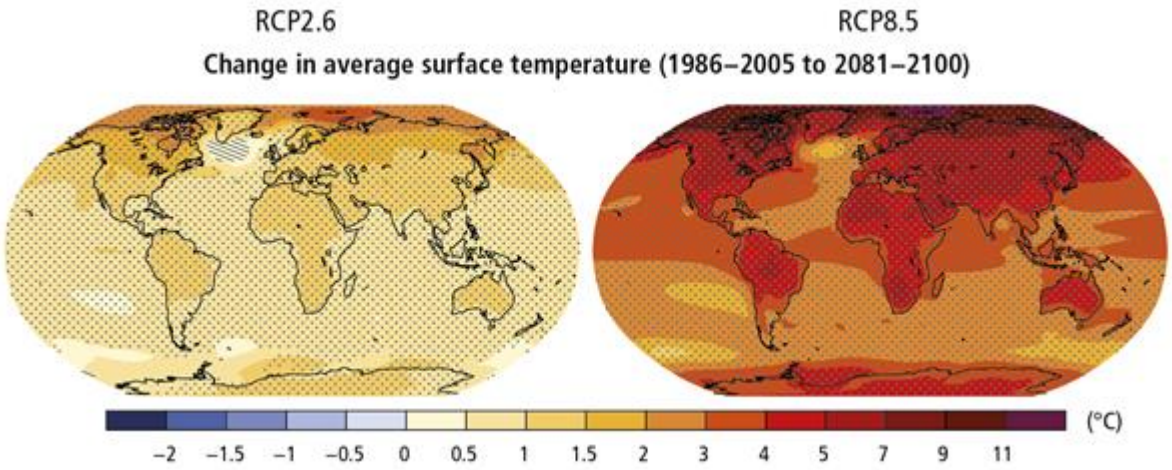

Figure 3.1 **Global warming.** Change in average surface temperature for 2081–2100 relative to 1986–2005 under the RCP2.6 (global mean increase of 1.0ºC) (left) and RCP8.5 (global mean increase of 3.7ºC) (right). Representative Concentration Pathways (RCPs) are four greenhouse gas concentration trajectories adopted by the IPCC. RCP2.6 assumes that global annual greenhouse gas emissions (measured in CO2-equivalents) peak between 2010-2020, with emissions declining substantially thereafter. In RCP8.5, emissions continue to rise throughout the 21st century. scenarios. Source: IPCC 2014.

1.3 BIOLOGICAL INVASIONS IN FUTURE ALTERED CLIMATES

Global warming is projected to strongly mediate biological invasions in freshwater ecosystems and is expected to intensify the threats to freshwater fauna, with higher temperature and more severe droughts modifying thermal and flow regimes, promoting the development of reservoir and canal systems to meet the growing human demand for freshwater (Sala et al 2000, Rahel et al 2008), and thus leading to the expansion in range and population size of warm-water invaders (Rahel & Olden 2008).

Temperature-specific competition, a process by which competition and dominance relations between species is mediated by temperature, is widespread in nature (i.e. Oyugi 2012a, 2012b, Taniguchi et al 1998) and may be of particular importance when studying the impacts of invasive species on natives in freshwater ecosystems, in future altered climates.

1.4 REGIONAL PERSPECTIVE

The freshwater ecosystems of the Iberian Peninsula harbour a rich and highly endemic freshwater fish fauna (Reyjol et al 2007), with many species restricted to individual rivers, streams, springs, wetlands and lakes across the region (Darwall et al 2014). A large proportion of these species is currently of major conservation concern (Smith & Darwall 2006), and highly threatened by habitat degradation and fragmentation and biological invasions (Allan and Flecker 1993; Collares-Pereira & Cowx 2004).

Iberian native freshwater fish are highly sensitive to the impacts of invasive species, with this impact considered as the leading cause of native fish decline (Hermoso et al 2011). Indeed, Iberian fresh waters are among the most heavily invaded systems in the world (Leprieur et al 2009, Clavero 2011), with new fish species being increasingly introduced (e.g. Franch et al 2008, Gante et al 2008), and previously established species expanding their ranges (e.g. Vinyoles et al 2007; Ribeiro et al 2009). Clavero and García-Berthou (2005) found that the total number of introduced species in Iberian basins increased

from 16 in 1991, to 22 in 1995, and 33 in 2001 (Figure 1.2) and that, in this 10-year period, main river basins had, on average, more invasive than native species.

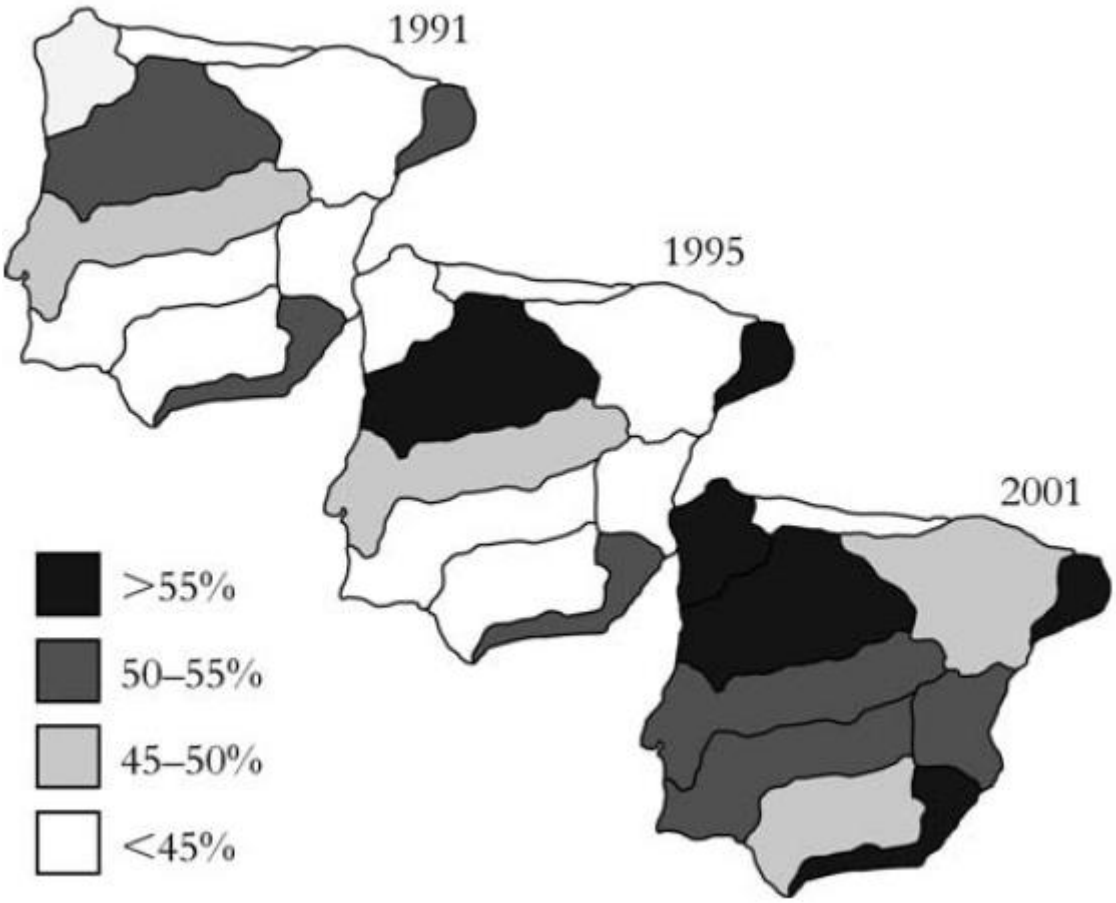

Figure 1.2 Variation of the percentage of introduced freshwater fish species in Iberian basins between 1991 and 2001. Adapted from Clavero and García-Berthou (2006).

The Iberian Peninsula, is potentially susceptible to significant climatic changes, with simulations for the region compounding the already dry and hot summers, and severe droughts (Giorgi & Lionello 2008), with forecasted substantial drying and warming of the region, especially in the summer, resulting in increases in inter-annual variability of the occurrence of extreme heat and drought events, and an average warming of around 5ºC, relative to 1986-2005, and potentially exceeding 7°C (Giorgi & Lionello 2008, IPPC 2013) (Figure. 1.3).

In this context, it becomes important to investigate how future global warming may interact with biological invasions to affect the diversity of freshwater endemic fish to the Iberian Peninsula. In particular, it will be important to understand to what extent increased temperatures will affect interactions between invasive and native fish, and whether that could result in changes in competitive dominance between species. Indeed, clarifying if predicted climate warming could magnify the impacts of non-natives on native species would be critical to forecast potential future trends in biological invasions and biodiversity loss.

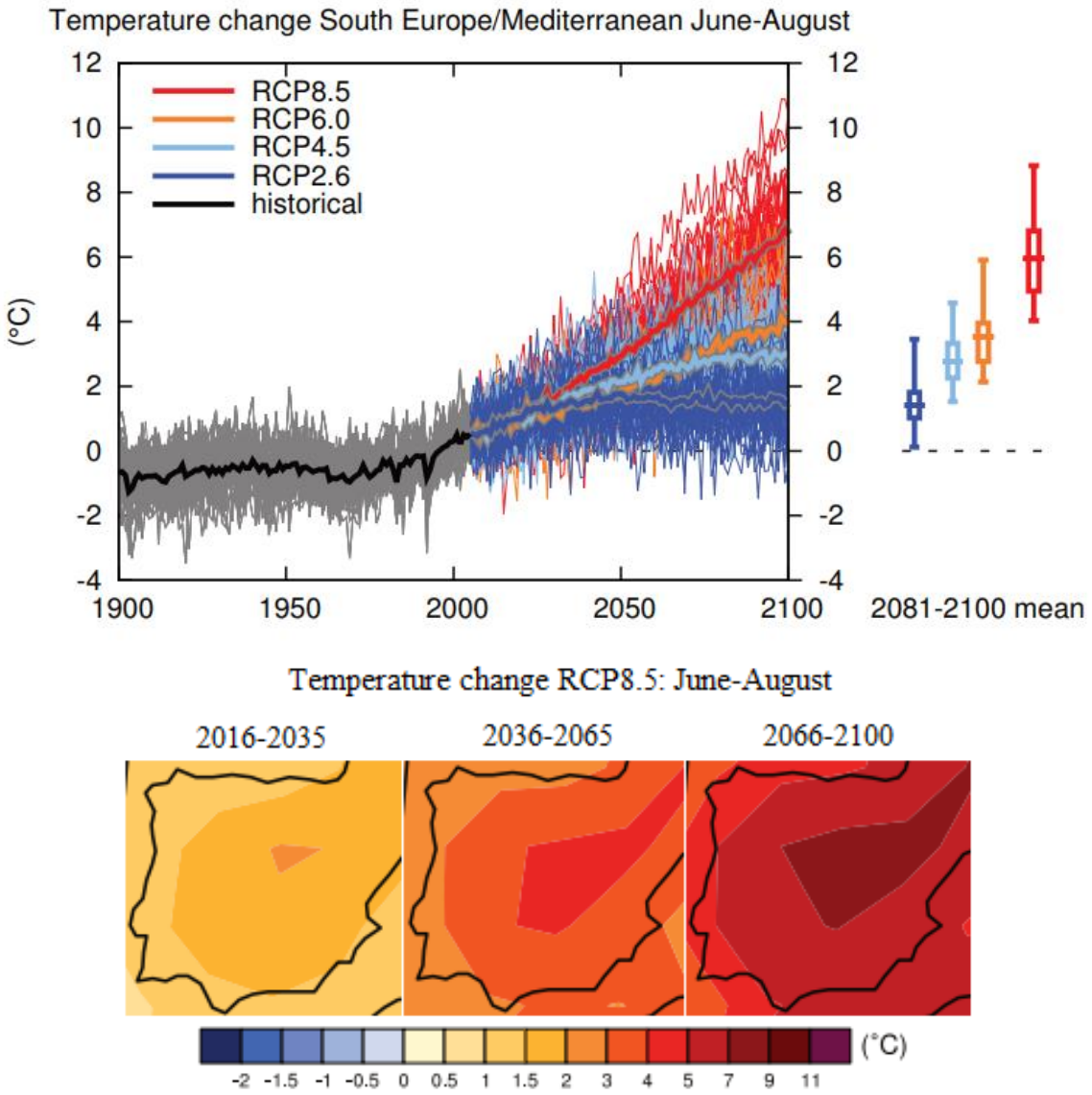

Figure 1.3 **Regional Warming.** (Top) Time series of temperature change relative to 1986–2005 averaged over land grid points in the region South Europe/Mediterranean in June to August. (bottom) Maps of temperature changes in 2016–2035, 2046–2065 and 2081–2100 with respect to 1986–2005, for June, July and August, in the RCP8.5 scenario. Adapted from IPPC (2013).

1.5 OBJECTIVES

The objective of this thesis was to evaluate the combined effects of temperature and the presence of invasive fish species on the foraging success of native Iberian freshwater fish. Specifically, using an experimental approach, we assessed: (i) the effects of the presence of two ecologically damaging invasive fish, the pumpkinseed sunfish *Lepomis gibbosus* (Linnaeus, 1758), native to the North American Great Lakes, and the chameleon cichlid *Australoheros facetus* (Jenyns, 1842), natural to the warm waters of South America, on the foraging success of the endemic Southern Iberian chub *Squalius pyrenaicus* (Günther, 1868), and (2) how this effect can be mediated by temperature.

Results were used to explore how competition between invasive and native species may be influenced by increased water temperatures, and to derive some recommendations regarding the control of invasive species and the conservation of native endangered species.

2. MATERIALS AND METHODS

2.1 MODEL SPECIES

Species used in this study were selected to represent typical Iberian native fish and damaging invasive fish with potentially variable temperature tolerances that could influence the outcomes of competition for food resources.

The Southern Iberian chub *Squalius pyrenaicus* (Günther 1868) is one of the most common cyprinids in the Iberian Peninsula (Blanco-Garrido et al 2003), occurring in the central and southern catchments of Iberia (Doadrio 2001). It is classified as "Near Threatened" in the European Red List of Freshwater Fishes (Freyhof & Brooks 2011) and included on Appendix III of the Bern Convention, with listed threats to this species including the introduction of non-native species (Leunda 2010). The chub can be found in small and large rivers and intermittent streams, well oxygenated, and with aquatic vegetation and shade (Magalhães 1993; Pires et al 1999). Like most Iberian species, it is a habitat generalist, and can tolerate a range of habitat conditions, using both pools and runs as summer refugia (Magalhães 1993, Coelho et al 1997, Blanco-Garrido et al 2003, Magalhães 2002). It may attain up to 210 mm in total length (TL) (Coelho et al 1997), reach maturity in its third year, at around 100 mm TL (Lobon-Ceryia 1982), and display considerable variation in diet throughout ontogeny, shifting from soft-bodied to hard-shelled prey and decreasing animal prey breadth (Magalhães 1993).

The centrarchid pumpkinseed sunfish *Lepomis gibbosus* (Linnaeus 1758), native to the temperate waters of the Great Lakes of North America, is one of the most invasive fish in Europe (Fox & Copp 2014), with a broad introduced range which extends from southern Norway to the Mediterranean region (Copp & Fox 2007, Cucherousset et al 2009). First introduced in the east of the Iberian Peninsula in the early 20th century, it only appeared in waters to the west in the 1970s (Sostoa et al 1987), quickly becoming a major, ecologically damaging invader (García-Berthou & Moreno-Amich 2000). The high success of pumpkinseed as an invader has been related to their high plasticity in life-history in both native and introduced areas (Copp & Fox 2007, Fox et al 2007, Fox & Copp 2014). Pumpkinseed sunfish attains a maximum size of 165 mm TL (Copp and Fox 2007), and it matures at 71 mm TL (Ribeiro & Collares-Pereira 2010). The pumpkinseed has a wide temperature tolerance, only ceasing feeding at 8.5ºC (Keast 1968) and, in its native range, can spawn in water that reach 27.8ºC (Scott and Grossman 1973). In its introduced range, where it occurs primarily in large pools (Magalhães 2002), the most common impact of pumpkinseed is its aggressive behaviour and diet overlap with native species, due to its generalist diet, that could affect foraging success of native species (García-Berthou & Moreno-Amich 2000, Godinho & Ferreira 2014, Almeida et al 2014).

The chameleon cichlid *Australoheros facetus* (Jenyns 1842), native to Paraguay, Uruguay, Argentina and Brazil (Říčan & Kullander 2006), where it is common in creeks, rivers, swamps and lakes (Říčan & Kullander 2008), was introduced to the Iberian Peninsula in 1940 (Doadrio 2001). This species is now invasive in drainages in both southern Portugal and Spain (Doadrio 2001; Collares-Pereira et al 2000). Reaching a maximum of 122 mm SL (Ribeiro et al 2007, Gonzalez Naya et al 2012), it has a generalist and highly flexible diet, consuming locally abundant food resources, and is thought to compete for food resources with the native fauna (Ribeiro et al 2007, Leunda 2010). In its invasive European range, the chameleon cichlid can tolerate a very wide range of temperatures, from almost freezing, to over 30ºC, at which it spawns (Kottelat & Freyhof 2007). Baduy et al (2016), have found that its critical thermal maximum and minimum, after specific acclimatization temperatures, are 39.1ºC and 4.5ºC, respectively.

Figure 2.1 **Model species.** Southern Iberian chub (top), *Squalius pyrenaicus,* pumpkinseed sunfish (bottom-left), *Lepomis gibbosus,* and chameleon cichlid (bottom-right), *Australoheros facetus*.

2.2 FISH SAMPLING

Fish were sampled between January 2016 and May 2017, in the Sado basin in southeast Portugal (Figure 2.2), where the three model species have been previously recorded (APA 2016). The Sado river drains about 7 692 km^2, originates from the Serra da Vigia and flows for 180 km, first from south to north and then north-east, into the Atlantic Ocean at Setubal. The Sado is the basin with the largest area completely in within Portugal, and presents a typical Mediterranean climate, with a strong seasonal pattern of hot and dry summers and mild and wet winters, leading to flooding and droughts. (APA 2016).

Fish sampling was conducted in two tributaries of the Sado river, that spring from the Serra de Grândola, selected because of the relative abundance of each species, ease of access and proximity to each other and to the laboratory facilities of FCUL. The chubs were caught in the São Martinho stream (38º10'09´´N, 8º34'10´´W) and both non-native species were caught in the Corona stream near Minas do Lousal (38°01'36´´N, 8°25'52´´W) (Figure. 2.2). These sites were chosen because model species were sufficiently abundant therein to yield samples for experiments, and local habitats were generally representative of natural and human-altered conditions found in Iberian streams. Both streams were bordered by pastoral lands, but whilst the S. Martinho stream had a healthy riparian zone, with many mature trees and shrubs, and a natural flow regime, the Corona stream had almost no riparian cover and was subjected to regularization of the river banks and bed.

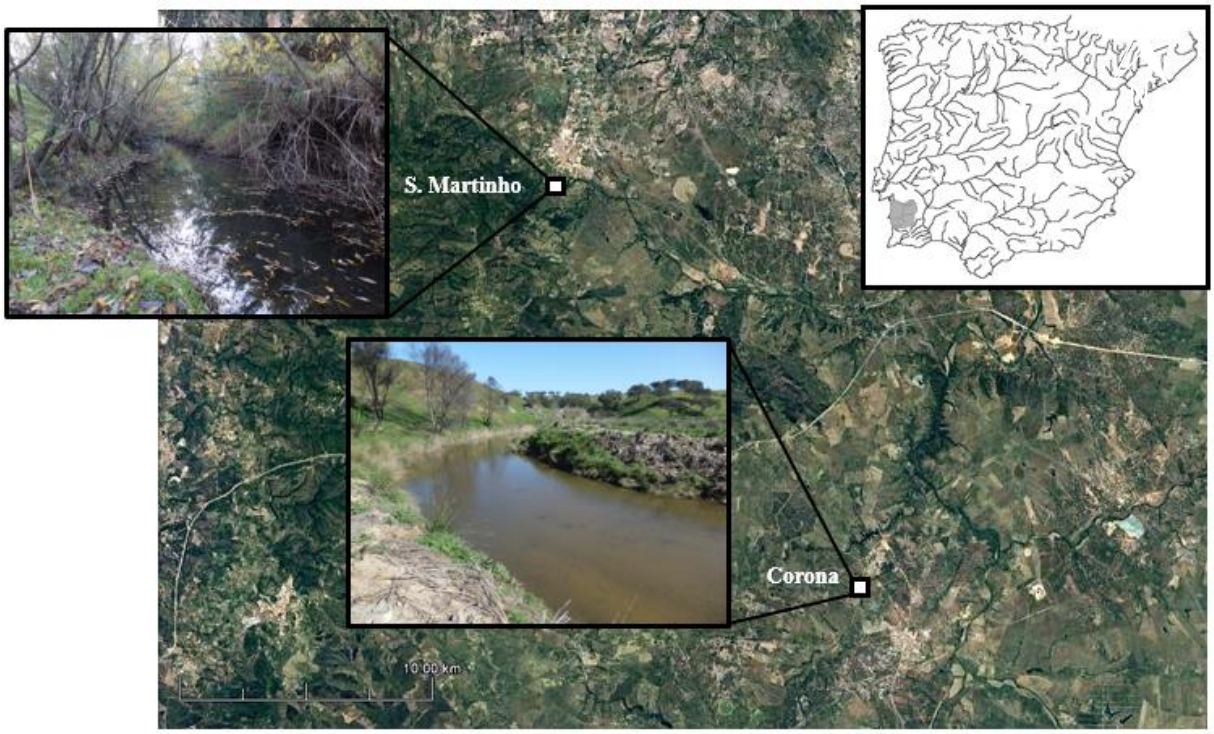

Figure 2.2 **Map of sampling sites.** Location of the sampling sites within the Sado river (inset), S. Martinho and Corona, where the chubs, and pumpkinseed and chanchito, were sampled, respectively.

Fish were sampled by electrofishing (Hans Grassl EL 62 generator DC, 300-600 V, 2–3 A, DC), and measured for total length, to the nearest mm. Only individuals between 45-80 mm were retained for further analysis, because this was the overlapping size range of all three species, in the sample chosen sample sites and to avoid potentially confounding effects of size variation in foraging success (Nakano et al 1998). Individuals were inspected for overall condition, and those with no signs of physical malformation, deformities, and parasites were separated by species into coolers, and transported to the laboratory. The remaining native and non-native fish were returned to the stream or euthanized, accordingly with Portuguese legislation.

Table 2.1 **Number and size of fish.** Number and total length (mm) of fish sampled in the Sado and used in foraging experiments

Species	Code	Number	Total length (mm)	
			Mean ± SD	Range
S. pyrenaicus	*Spyr*	99	63. 9 ± 8.0	45 - 80
L. gibbosus	*Lgib*	43	62.2 ± 6.9	48 - 76
A. facetus	*Afac*	44	60.2 ± 7.5	48 - 75

Up to 25 individuals of each species were retained at any one sampling event, due to space and resource constraints in transport and laboratory conditions. Overall, 186 individual fish (99 chubs, 43 pumpkinseeds and 44 chameleon cichlids) were retained for experimental trials (Table 2.1).

2.3 FISH HANDLING AND ACCLIMATIZATION

Upon reaching the lab, room-temperature water (14-22ºC) from the acclimatization aquaria was slowly added to each cooler, until they were at the same temperature, and the fishes could be transferred to separate quarantine aquaria, to acclimatize to the laboratory conditions and to evaluate if fish did not carry any disease or parasite. This process took approximately 30 minutes, in order to reduce physiological stress to the animals and ensure a good animal welfare.

A total of 19 aquaria ranging from 10 to 90 L, were used for (i) quarantine proposes (two 90 L and one 40 L), (ii) acclimatization to temperature treatments (fifteen 10-30L) and (iii) to conduct experiments (one 30 L). All aquaria were prepared at least 48 hours in advance to fish sampling to allow chlorine from the tap water to evaporate. The two 90 L aquaria, contained a layer of filter wool and bio-balls for physical and biological filtration below a hard, lightly perforated, hard plastic sheet, on to which fine gravel and some small (3-5 cm) and large (10-15 cm) stones were placed, to counteract the buoyancy of the filtering layer and to simulate a natural environment, providing refuge for fish and diminishing stress during acclimatization. Each aquaria had two air pumps (MARINA 70), with diffusers placed in PVC tubes, that vertically passed through the plastic sheet, from just under the water line, through to the filtering elements below, to improve oxygenation and to pull water and detritus down and through the wool. Two water filters (EHIEM) were placed at opposite ends of the aquaria, each with sponges and bio-balls, with the water exiting just above water level, to create a soft cascading flow (Figure. 2.3). The remaining aquaria (10-40L), had water filters (ELITE STINRAY 5/10) filled with bio-balls and sponges, and small stones (3-5 cm) on the bottom. To avoid excess build-up of organic waste in these smaller aquariums, water was periodically siphoned and replaced with water kept in 5 L open bottles at room temperature to dechlorinate (Figure. 2.3). All aquaria were placed on tables (90 L) or metal shelves (<90 L) with 2 cm thick Styrofoam, to minimize the propagation of vibrations from the water filters (Figure. 2.3). Fish density in acclimatization aquaria was never greater than 1 individual per 3 L.

After 2 weeks of quarantine and acclimatization to laboratory conditions, fish were placed in temperature acclimatization aquaria, separated by species, experimental temperature and experiment date. Pairs of fish to be used in paired experiments (see below) were selected prior to temperature acclimatization, so that the maximum difference in size between individuals in each pair were less than 10 mm TL, to minimize the potentially confounding effect of size on foraging success. Fish were subjected to a temperature adjustment procedure of progressive modifications of water temperature, of 1-2ºC per day, and maintained at the desired experimental temperature treatment for at least 7 days before the start of experimental trails.

Temperatures were adjusted daily using thermostats (BOYU HT-850) and monitored with a temperature probe (WTW LF197), until the experimental temperatures were achieved (19, 24 or 29, ±0.5ºC, see below). Fish were fed frozen "*Chironomid* larvae" daily, thawed out and delivered by pipette, and kept at a 12/12h photoperiod. Prior to trials fish were starved for at least 48 hours, to encourage feeding and to ensure that all specimens experienced similar hunger levels.

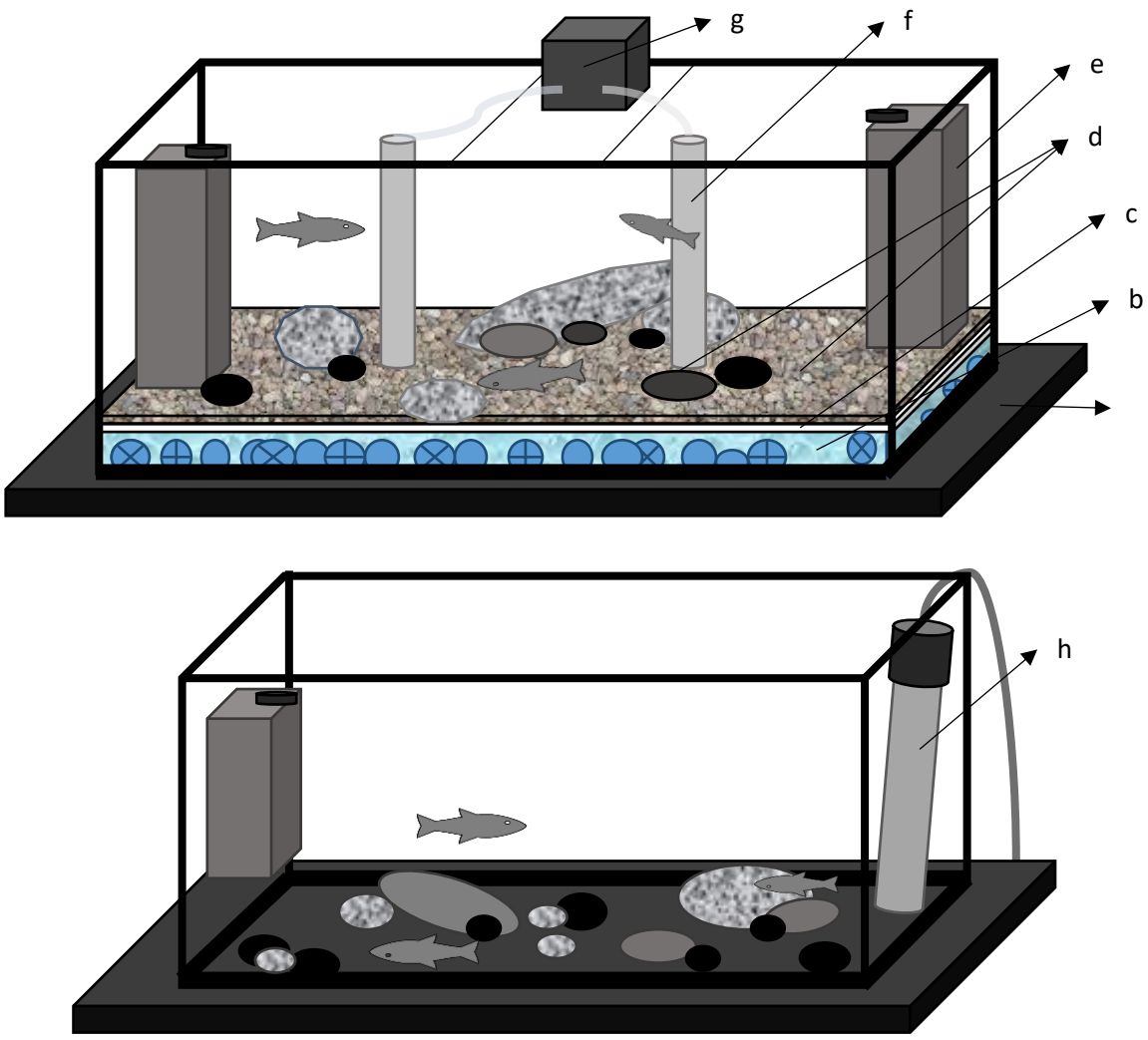

Figure 2.3 **Structure of quarantine and acclimatization aquaria.** Structure of the of the 90 L quarantine aquaria (top) and of the 10 to 40 L acclimatization aquaria (bottom): a) Styrofoam covered in black plastic; b) layer of filter wool and bio-balls; c) hard plastic sheet; d) fine gravel and/or stones; e) water filter; f) PVC tube containing clear plastic tube and diffuser; g) air pump; h) thermostat.

2.4 EXPERIMENTAL DESIGN

Experimental trials were designed to test for the effect of temperature on the foraging success of each species when on its own and on foraging success of chub when in the presence of another species, either another chub or each of the invasive species.

2.4.1 Densities

The experiments included three single-species groups – (1) *Squalius pyrenaicus*, (2) *Lepomis gibbosus* and (3) *Australoheros facetus*; one intraspecific paired-species groups – (4) *Squalius pyrenaicus vs Squalius pyrenaicus*; and two interspecific paired-species groups – (5) *Squalius pyrenaicus* vs. *Lepomis gibbosus*, (6) *Squalius pyrenaicus* vs. *Australoheros facetus.*

2.4.2 Temperature

Because water temperature at the sampling sites throughout the sampling period ranged from 10 to 24ºC (Table S3.1) and the expected increase in air temperature, over the next century, for the region

encompassing the Sado basin is of around 5ºC (Figure 1.2), feeding trails were conducted at 19, 24 and 29ºC (i.e. 24ºC±5ºC). These three experimental temperature treatments were assumed to represent at least partially the range of conditions in future climate warming in the Sado region.

Overall, to test each group at each temperature treatment, we conducted a total of 18 trials (6 species groups x 3 temperature treatments), each of which was replicated between 6 to 9 times (Table 2.2).

Table 2.2 **Number of replicates of trials.** Description of the species groups, temperature treatments, and number of replicates used in feeding trails (species group x temperature treatment)

Group	Combination	Species 1	Species 2	Nº of Replicates Per Temperature Treatment		
				19	24	29
1	Single	*S. pyrenaicus*	-	6	7	6
2	Single	*L. gibbosus*	-	9	7	7
3	Single	*A. facetus*	-	8	8	8
4	Intraspecific	*S. pyrenaicus*	*S. pyrenaicus*	6	7	7
5	Interspecific	*S. pyrenaicus*	*L. gibbosus*	6	7	7
6	Interspecific	*S. pyrenaicus*	*A. facetus*	6	7	7
			Total	41	43	42
					126	

2.5 FEEDING TRIALS

Feeding trails were conducted in a 30 L aquaria, divided in two equal compartments by a removable plastic mesh barrier, with white plastic attached to the back and sides. Five clear plastic tubes (5 mm ⌀) placed at the corners and back of the aquaria, just below the water level to minimize disturbance, allowed us to deliver the prey, propelled by a small amount of water at the top of the tube, attached to the upper shelve, using a 20 cl syringe (Figure. 2.4). In both single and paired trials, each fish was placed in each side of the barrier, randomly, and allowed 10 minutes to recover from handling before the experiment began.

Feeding trails were videotaped (SONY HANDYCAM DCR-SR32), at a distance of 50 cm from the front of the experimental aquaria, always by the same operator. The operator was located just behind the aquaria and not visible to the fish during experiments, due to the plastic on the sides and back of the aquaria. Each trial lasted about 20 minutes, beginning immediately before the plastic barrier between compartments was gently raised to the surface and removed. Each trial consisted of 10 deliveries of single *Chironomidae* frozen larvae, at a minimum of two-minute intervals, through one of the five tubes, randomly selected in advance, through a random number generator. Larvae of Chironomidae were selected for trials as well as for daily feeding of fish during acclimatization, (see above), because they are an important prey of all model species in the wild (Blanco-Garrido et al 2003, Godinho & Ferreira 2014, Ribeiro et al 2007), and frozen individuals are non-mobile and highly visible (bright red) which facilitated videotaping and observation. Each individual fish was only used in one trial. After each experiment, non-native pumpkinseed and chameleon cichlid were euthanized using MS222, and chubs were moved to a post-experiment holding aquaria (50 L), slowly acclimated to room temperature as described above, and later returned to the sites of collection.

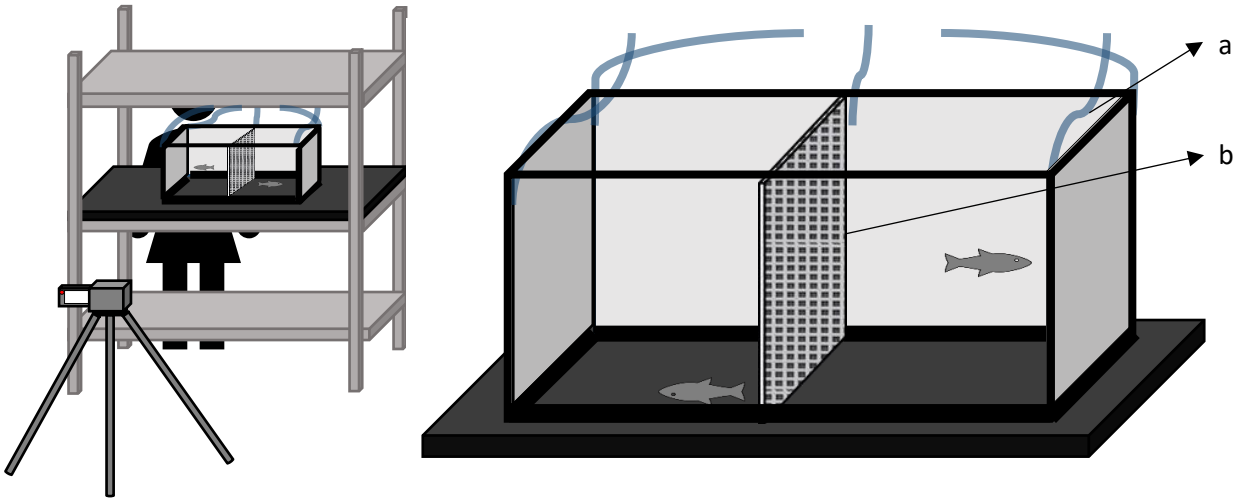

Figure 2.4 **Structure and position of experimental aquaria.** Position of video recorder and operator, relative to experimental aquaria (left) and the 30 L experiment aquaria (right): a) tubes for delivering preys during experiments; b) plastic mesh barrier to separate pairs of fish prior to intra and interspecific competition experiments.

2.6 METRICS AND DATA ANALYSIS

Foraging success was primarily derived from the number of prey captured by each fish in each trial. Single and paired species trials were analysed separately, because fish density differed from 1 to 2 fish, respectively, but the number of preys released was constant meaning that the probability that an individual will capture a prey will decrease with density.

For single species trials, comparisons of foraging success were made among temperature treatments for each species group, thus testing for the effect of temperature on the foraging success of individual species, and for the same temperature among species groups, there by testing variation in foraging success among species. For paired species trials, we compared the foraging of the same pair of species among temperatures, thus assessing the effects of temperature on the foraging success of the chub when in the presence of another chub (intraspecific competition) or of either of the two invasive species (interspecific competition). We additionally compared the foraging success among species pairs, at the same temperature, to evaluate for chub's variation in this metric, when in the presence of either another chub or one of the invasive species, across those temperatures. In paired experiments, the trial codes to be used henceforth (e.g. *SpyrSpyr*19 vs *SpyrLgib*19 vs *SpyrAfac*19) indicate the species that are being tested and the temperature treatment (in ºC) (Table 2.3).

Table 2.3 **Trial codes.** Comparisons made among species and temperatures treatments in single and paired species experiments

Single Experiments	Paired Experiments
*Spyr*19 vs *Spyr*24 vs *Spyr*29	*SpyrSpyr*19 vs *SpyrSpyr*24 vs *SpyrSpyr*29
*Lgib*19 vs *Lgib*24 vs *Lgib*29	*SpyrLgib*19 vs *SpyrLgib*24 vs *SpyrLgib*29
*Afac*19 vs *Afac*24 vs *Afac*29	*SpyrAfac*19 vs *SpyrAfac*24 vs *SpyrAfac*29
*Spyr*19 vs *Lgib*19 vs *Afac*19	*SpyrSpyr*19 vs *SpyrLgib*19 vs *SpyrAfac*19
*Spyr*24 vs *Lgib*24 vs *Afac*24	*SpyrSpyr*24 vs *SpyrLgib*24 vs *SpyrAfac*24
*Spyr*29 vs *Lgib*29 vs *Afac*29	*SpyrSpyr*29 vs *SpyrLgib*29 vs *SpyrAfac*29

Two metrics were used to derive fish foraging success:

1) Number of Prey Captured (NPrey) was determined from the number of prey captured by each fish (range: 0-10 preys), with a capture representing a fish grasping a prey, independent of whether the prey was consumed or not, following Hazelton & Grossman (2009).
2) Mean Time to Capture Preys (mTCap) defined as the number of seconds between a prey being released into the water and being captured by a fish, before a new prey was released (range: 0-120 seconds).

Variation in the number of prey captured and time to capture prey was tested using the non-parametric Kruskal-Wallis H test (Kruskal & Wallis 1952), and post-hoc two-tailed Dunn's Multiple Comparison Test (Dunn 1964). For both metrics, trials with less than 5 observations were discarded from analysis. Additionally, we tested for variation in fish total length across trials, to assess whether this variable had been successfully controlled for during the pre-experimental procedures, and ensure there were no potentially confounding effects of individual size advantages. Construction of the database and statistical analysis were conducted using the software Python, version 2.7 (Python Software Foundation, https://www.python.org/), and significance of statistical testing was assessed at $p<0.05$.

3. RESULTS

3.1 INDIVIDUAL SPECIES FORAGING

There were no significant differences in the size (total length) of fish used in single species trials neither among species nor among temperature treatments (see Table S1.1).

3.1.1 Number of Prey Captured

The Number of Prey Captured by chub was not significantly different among treatment temperatures (Table S1.1), with average values ranging between 3.3 to 4.7 (range: 0-10) (see Table S2.3). Conversely, pumpkinseed captured significantly less prey at 24ºC (average: 5.6; range 1-9) than at 29ºC (average 9.3, range: 6-10) (p=0.006) (Table S1.1). Likewise, chameleon cichlid showed a slight tendency (p=0.051) to capture less prey at 19ºC (average 6.6; range 0-10), than at the two higher temperatures both at which it captures an average of 9.5 or more preys (range: 8-10) (Table S1.1, Table S2.3).

No significant difference in Number of Prey Captured among species was found at 19ºC. However, at 24ºC, chameleon cichlid captured significantly more prey than both chub (p=0.002) and pumpkinseed (p=0.009), and at 29ºC, chub captured significantly less prey than pumpkinseed (p=0.005) and chameleon cichlid (p=0.003) (Table S1.1) (Figure 3.1).

3.1.2 Mean Time to Capture Prey

Mean Time to Capture Prey was not assessed for chub at 19ºC due to too few observations (N<5).

Chub displayed no significant difference in the time to capture prey between 24 and 29ºC (Table S1.1). Likewise, no variation was found for pumpkinseed among the three temperature treatments (p=0.058), although it showed a slight tendency to capture prey slower at 24ºC (Table S1.1). Conversely, chameleon cichlid captured prey significantly faster at the two higher temperature treatments, than at 19ºC (*Afac*19 vs *Afac*24: p=0.006, *Afac*19 vs *Afac*29: p=0.003) (Table S1.1), at which it takes roughly five times longer to capture preys (see Table S2.5).

At 19ºC, the invasive species captured prey at a similar speed (Table S2.5). At 24ºC, chameleon cichlid captured prey much faster (i.e. about 30 times faster) than chub (p=0.001) (Table S1.1). At 29ºC, both invasive species capture prey over 10 times faster than the native chub (*Spyr*29 vs *Lgib*29: p=0.009; *Spyr*29 vs *Afac*29: p=0.003) (Table S1.1) (Figure 3.1).

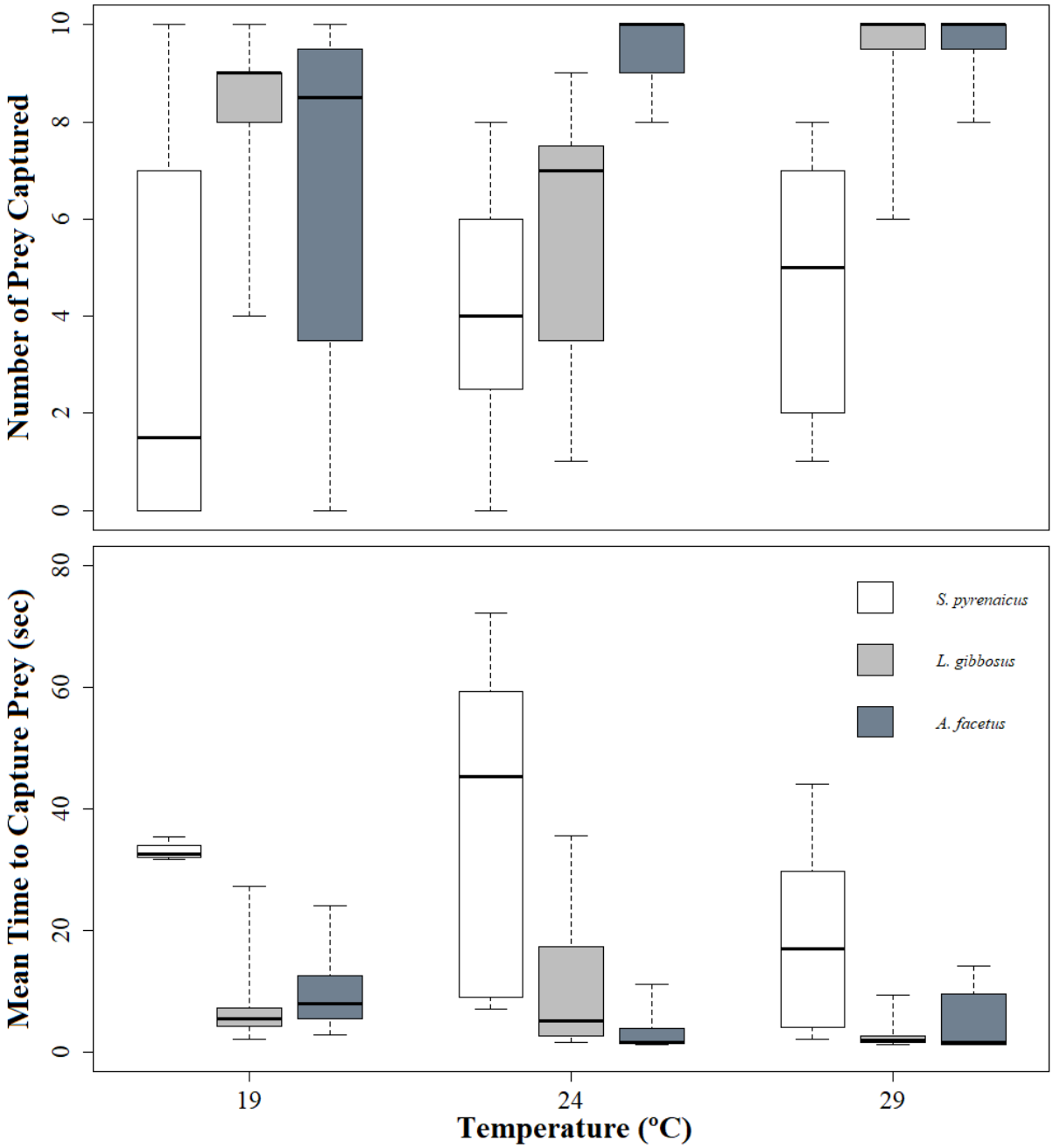

Figure 3.1 **Results of single species trials.** Boxplots of Number of Prey Captured (above) and Mean Time to Capture Prey (bellow) in single species trials for each species and temperature treatment. Dark line indicates the median, and whiskers the minimum and maximum values.

3.2 FORAGING IN SPECIES PAIRS

There were no significant differences in the size (total length) of fish used in paired trials neither among species nor among temperature treatments (Table S1.2). Difference in size between fish in pairs was on average 2.75 mm and at most 8 mm (Table S2.2).

3.2.1 Number of Prey Captured

The Number of Prey Captured by chub when in paired species group across the different temperatures, was not significantly different (Table S1.2).

At 19ºC, chub captured significantly more preys when in the presence of another chub than when with chameleon cichlid (p=0.037) (Table S1.2). At 24ºC, chub captured significantly more prey, both when with another chub and when with pumpkinseed, than when with chameleon cichlid (*SpyrSpyr*24 vs *SpyrAfac*24: p=0.008; *SpyrLgib24* vs *SpyrAfac*24: p=0.012), in which trials chub failed to capture a single prey (Table S1.2). At 29 ºC, chub captured significantly more prey when with another chub than when with either of the invasive species (*SpyrSpyr*29 vs *SpyrLgib*29: p=0.049; *SpyrSpyr*29 vs *SpyrAfac*29: p=0.006). (Table S1.2) (Figure 3.2).

3.2.2 Mean Time to Capture Prey

Due to too few observations Mean Time to Capture Prey could not be assessed for chub when with chameleon cichlid at any temperature and when with pumpkinseed at 29ºC.

There were no significant differences at 19, 24 and 29ºC in the mean time it took chubs to capture prey, when in the presence of another chub (9.2, 23.8 and 5.8 seconds, respectively) (Table S1.2 and Table S2.6). When with pumpkinseed, the time it took chub to capture prey was similar at 19 and 24º C (2.3 and 3.7 seconds, respectively) (Table S2.6). When competing with chameleon cichlid at 19 and 29ºC, the time it took chub to capture prey was also similar (31.2 and 27.6 seconds, respectively) (Table S2.6).

When comparing among species-groups at each temperature, at 19ºC, chub captured prey faster when with pumpkinseed (2.3 seconds) than when with a conspecific (9.2 seconds), but three times slower when with chameleon cichlid (31.2 seconds) (Table S2.6). At 24ºC it was significantly faster when with pumpkinseed, than with another chub (p=0.013) (Table S2.6). At 29ºC, like at the lowest temperature, chub was fastest when competing with pumpkinseed (2.1 seconds), and slowest when with chameleon cichlid (27.3 seconds), compared to when with another chub (9.2 seconds) (Table S2.6) (Figure 3.2).

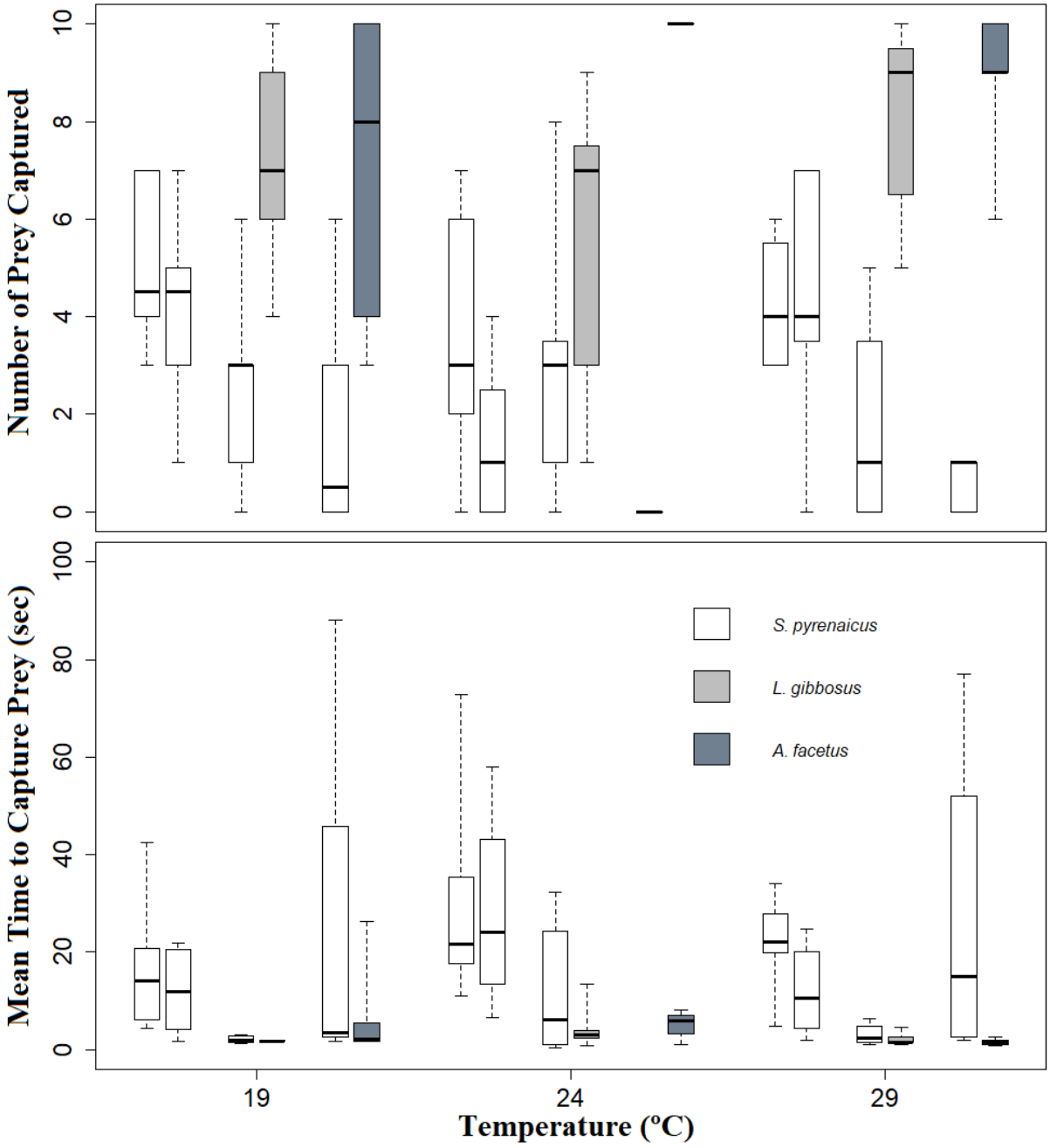

Figure 3.2 **Results of paired species trials.** Boxplots of Number of Prey Captured (above) and Mean Time to Capture Prey (bellow), for each paired species trial. Dark line indicates the median value, whiskers represent the minimum and maximum values, paired species groups in order, for each temperature, from left to right: *SpyrSpyr*; *SpyrLgib*, *SpyrAfac*.

4. DISCUSSION AND CONCLUSIONS

Although the underlying mechanisms involved in the interactions between native and non-native species are often ambiguous (Leunda 2010), experimental approaches have previously demonstrated that invasive fishes affect the foraging success of native species (i.e. Caiola & de Sostoa 2005; Alcaraz et al 2008; Hazelton & Grossman 2009) and competition for food between invasive and native species is particularly important, with cascading effects potentially disrupting ecosystem function and food webs (Baxter et al 2004). When individuals simultaneously encounter a single prey item, those that capture more and faster, thus with better competitive abilities, can successfully obtain prey at the expense of a reduction in foraging success of the other competitors (Milinski and Parker 1991, Gill & Hart 1996). This kind of competition is expected to become more likely when subjected to high temperatures, resulting in strong biological interactions between organisms (Gasith & Resh 1999, Pires et al 2000), as demonstrated in this study.

This study provides evidence of the differing foraging capabilities of the Southern Iberian chub, the pumpkinseed sunfish and the chameleon cichlid, mediated by a range of temperatures that may be expected to occur in Southern Iberian Peninsula by the end of the 21st century (IPCC 2013). Individually, both invasive species were more successful than the chub at capturing prey and did so 10 to 30 times faster, with this trend increasing with the rise in temperature. When competing, the native chub captured fewer prey than either invasive species, especially at warmer temperatures. The time it took chub to capture prey decreased when in the presence of pumpkinseed but greatly increased in the presence of the chameleon cichlid.

A potential limitation to our study was related to the capture of fish over the entire year (Table S3.1), because some fish could possibly have been captured during or close to their reproductive period, with unknown effects on foraging behaviour. Another possible confounding factor on the results was the presence of pumpkinseed in the S. Martinho river, from which the chubs were sampled. This previous exposure to the invasive species, could result in a more "practiced" foraging behaviour with this species than with the chameleon cichlid, and should be assessed further.

Temperature did not significantly affect the number of, or the speed with which, the chub captured prey, both when alone and when competing with a conspecific. This was expectable of a species that evolved in highly variable seasonal and inter-annual climatic conditions typical of the Iberian climate (Gasith & Resh 1999), and display good adaptability and resilience to sudden and unpredictable changes in abiotic conditions (Rodrigues 1999). However, chub tended to underperform, in terms of speed, at 24ºC and there was a slight trend indicating the increase in prey capture with the rise in temperature, when foraging alone. This tendency to capture more prey at higher temperatures, when not having to compete for this resource, likely reflects the increase in food consumption rate with water temperature until thermally stressful conditions are reached (Rahel & Olden 2008, Lang et al 2012).

The two invasive species have already been considered to be more successful foragers than the chub, because of their opportunistic life-history traits, due to the reduced predation risk relative to their native ranges and the absence of congeneric competitors in Iberian waters (Copp & Fox 2007, Fox et al 2007, Fox & Copp 2014, Masson et al 2014). Our results agree with this, with chub only capturing, on average across temperatures, about half as many prey as either invasive in single trials. In single trials, pumpkinseed showed a slight advantage over chameleon cichlid and more considerably so in relation to chub, at 19ºC, whereas at 24ºC chameleon cichlid captured more prey and did so faster than chub and

pumpkinseed. At 29ºC, pumpkinseed and chameleon cichlid were at their fastest and all species captured more prey than at the other temperatures. When in paired experiments, both invasive species outperformed the chub and captured more preys at all temperatures and chub took less time to capture preys when in the presence of the invasive species, than when competing with another chub. Whilst temperature did not affect the foraging success of chub across species groups, for each temperature treatment, foraging success varied depending on the species competing with the chub. At 19 and 24ºC, chub captured significantly less prey when competing with chameleon cichlid, than when with a conspecific, whereas at 29ºC this occurred when with either invasive species.

Therefore, we can conclude that the presence of both pumpkinseed and the chameleon cichlid has a negative effect on the foraging success of the native chub, one of the most common cyprinid species in the Iberian Peninsula, and that this effect is heightened by the increase in temperature when with chameleon cichlid, whilst interactions between chub and pumpkinseed are less accentuated and varied more across temperatures.

Overall, results from this study suggest that competition between native and invasive fish in Iberian freshwater ecosystems becomes more likely at the higher temperatures expected under future altered climates, with advantages for invaders. This could contribute to a rise in introduction and, higher rates of establishment and an increase in invasive species range, possibly leading to increasing risks of native species displacement, dietary shifts and biodiversity loss (Rahel & Olden 2008). Possible management measures to minimize these trends may include the renaturalization of the human-altered streams and recovery of riparian cover, to provide more appropriate habitats and temperature refuges for native species, but also the control of invasive populations, by regular extraction efforts, and increasing public awareness and monitoring activities, to not only prevent new species introductions but also the spread of existing ecologically damaging invasive species.

4.1 FINAL REMARKS

This study provided experimental evidence on the potentially detrimental effects of invasive species and climate change on native Iberian freshwater fish. To further understand the complex interactions that could threaten the Iberian freshwater fauna under future climates it will be important to further clarify drivers of foraging success of both native and invasive species, and specifically to:

- Test at different densities of fishes, to simulate more realistic competitive behaviours, and at other temperatures, to represent a wider range of possible future temperatures.
- Assess different sizes and ontogeny stages, given the diet shifts associated with this factor (Magalhães 1993, Gárcia-Berthou & Moreno-Amich 2000, Ribeiro et al 2007).
- Compare the effect of the presence of invasive species of fish with and without prior exposure to that species, to ascertain possible adaptive responses of natives.
- Evaluate competition-dominance relations between invasive species.
- Analyse the effects of other abiotic factors, such as flow and turbidity, that could alter foraging success (Sweka & Hartman 2001, Rincón et al 2007) and are also expected to vary with climate warming.

Clarifying multiple influences on fish foraging success will be critical to better predict the evolution of species interactions as the climate changes, and enhance our ability to manage and preserve Iberian freshwater ecosystems.

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APPENDIX I

Table S1.1 **Results of Kruskal-Wallis and Dunn's tests of single trials.** Results of Kruskal-Wallis and post-hoc Dunn's tests of all single species trials compared, for the variables Size (TL, mm), NPrey (Number of Prey Captured) and mTCap (Mean Time to Capture Prey). Significant p-values in bold

	Kruskal-Wallis Test						**Dunn's Test**			
Single	**Size**		**NPrey**		**mTCap**		**NPrey**		**mTCap**	
Spyr19 vs Lgib19 vs Afac19	W= 1.309	p= 0.520	W= 3.977	p= 0.137	W= 2.160	(1) p= 0.142	-	-	-	-
Spyr24 vs Lgib24 vs Afac24	W= 1.821	p= 0.402	W= 13.416	p= **0.001**	W= 12.321	p= **0.002**	Spyr24 vs Afac24 z= -3.429 p= **0.002** Lgib24 vs Afac24 z= -2.739 p= **0.009**		Spyr24 vs Afac24 z= 3.510 p= **0.001**	
Spyr29 vs Lgib29 vs Afac29	W= 3.836	p= 0.147	W= 12.556	p= **0.002**	W= 12.011	p= **0.003**	Spyr29 vs Lgib29 z= -2.953 p= **0.005** Spyr29 vs Afac29 z= -3.267 p= **0.003**		Spyr29 vs Lgib29 z= 2.745 p= **0.009** Spyr29 vs Afac29 z= 3.283 p= **0.003**	
Spyr19 vs Spyr24 vs Spyr29	W= 1.864	p= 0.394	W= 0.864	p= 0.649	W= 2.084	(1) p= 0.149	-	-	-	-
Lgib19 vs Lgib24 vs Lgib29	W= 0.530	p= 0.767	W= 9.585	p= **0.008**	W= 5.702	p= 0.058	Lgib24 vs Lgib29 z= -3.095 p= **0.006**		-	-
Afac19 vs Afac24 vs Afac29	W= 3.918	p= 0.141	W= 5.956	p= 0.051	W= 12.567	p= **0.002**	-	-	Afac19 vs Afac24 z= 2.875 p= **0.006** Afac19 vs Afac29 z= 3.288 p= **0.003**	

(1) Spyr19 discarded (observations<5)

Table S1.2 **Results of Kruskal-Wallis and Dunn's tests of paired species trials.** Results of Kruskal-Wallis and post-hoc Dunn's tests of all paired species trials compared, for the variables Size (TL, mm), NPrey (Number of Prey Captured) and mTCap (Mean Time to Capture Prey). Significant p-values in bold

	Kruskal-Wallis Test						**Dunn's Test**			
Paired	**Size**		**NPrey**		**mTCap**		**NPrey**		**mTCap**	
SpyrSpyr19 vs SpyrLgib19 vs SpyrAfac19	W= 0.586	p= 0.746	W= 6.658	p= **0.036**	W= 3.411	(2) p= 0.065	SpyrSpyr19 vs SpyrAfac19 z= 2.505	p= **0.037**	-	-
SpyrSpyr24 vs SpyrLgib24 vs SpyrAfac24	W= 2.70	p= 0.260	W= 10.892	p= **0.004**	W= 6.18	(4) p= **0.013**	SpyrSpyr24 vs SpyrAfac24 z= 3.021	p= **0.008**	SpyrSpyr24 vs SpyrLgib24 z= 2.486	p= **0.013**
							SpyrLgib24 vs SpyrAfac24 z= 2.661	p= **0.012**		
SpyrSpyr29 vs SpyrLgib29 vs SpyrAfac29	W= 0.422	p= 0.810	W= 9.98	p= **0.007**	-	(3) (5) -	SpyrSpyr29 vs SpyrLgib29 z= 2.137	p= **0.049**	-	-
							SpyrSpyr29 vs SpyrAfac29 z= 3.084	p= **0.006**		
SpyrSpyr19 vs SpyrSpyr24 vs SpyrSpyr29	W= 1.573	p= 0.456	W= 1.198	p= 0.549	W= 5.426	p= 0.066	-	-	-	-
SpyrLgib19 vs SpyrLgib24 vs SpyrLgib29	W= 1.081	p= 0.582	W= 0.664	p= 0.717	W= 0.211	(3) p= 0.646	-	-	-	-
SpyrAfac19 vs SpyrAfac24 vs SpyrAfac29	W= 0.002	p= 0.999	W= 5.335	p= 0.069	-	(2, 4, 5) -	-	-	-	-

(2) Spyr-Afac19 discarded (observations<5); (3) Spyr-Lgib29 discarded (observations<5); (4) Spyr-Afac24 discarded (observations<5); (5) Spyr-Afac29 discarded (observations<5)

APPENDIX II

Table S2.1 **Size of fish in single species trials.** Descriptive statistics for variable Size (TL, mm), of each single species trial

Single	Min	1st Q	Median	Mean	3rd Q	Max
Spyr19	45.00	50.50	55.50	56.50	59.75	73.00
Spyr24	48.00	50.00	52.00	56.71	62.50	72.00
Spyr29	54.00	55.75	62.00	62.33	67.50	73.00
Lgib19	50.00	51.00	54.00	59.44	67.00	74.00
Lgib24	51.00	56.50	62.00	62.29	67.00	76.00
Lgib29	53.00	57.50	63.00	61.00	64.50	67.00
Afac19	49.00	50.00	52.00	54.12	57.00	66.00
Afac24	49.00	58.25	64.50	61.00	65.25	68.00
Afac29	68.00	54.50	55.50	56.12	58.50	62.00

Table S2.2 **Size of fish in paired species trials.** Descriptive statistics for variable Size (TL, mm), of each paired species trial

Paired		Min	1st Q	Median	Mean	3rd Q	Max
SpyrSpyr19	**Spyr**	51.00	62.00	66.00	65.67	73.00	75.00
	Spyr	49.00	63.50	68.00	67.67	76.25	80.00
SpyrSpyr24	**Spyr**	59.00	63.50	70.00	68.43	74.00	75.00
	Spyr	61.00	66.00	67.00	68.14	70.50	76.00
SpyrSpyr29	**Spyr**	59.00	61.00	65.00	64.86	68.50	71.00
	Spyr	57.00	60.50	66.00	65.43	69.00	76.00
SpyrLgib19	**Spyr**	56.00	64.75	68.50	67.17	70.75	75.00
	Lgib	53.00	67.00	67.50	66.83	70.25	75.00
SpyrLgib24	**Spyr**	54.00	59.50	67.00	64.14	68.00	73.00
	Lgib	59.00	61.50	65.00	64.00	66.00	69.00
SpyrLgib29	**Spyr**	58.00	62.00	64.00	64.29	66.50	71.00
	Lgib	63.00	64.50	65.00	66.14	67.00	72.00
SpyrAfac19	**Spyr**	49.00	53.00	61.50	62.00	70.00	77.00
	Afac	48.00	51.50	60.00	60.00	67.00	74.00
SpyrAfac24	**Spyr**	54.00	60.00	63.00	62.71	64.50	73.00
	Afac	53.00	60.50	66.00	63.71	68.00	70.00
SpyrAfac29	**Spyr**	48.00	55.50	67.00	61.71	68.00	70.00
	Afac	48.00	54.50	61.00	60.00	66.00	70.00

Table S2.3 **Number of Prey Captured in single species trials.** Descriptive statistics for variable Number of Preys Captured of each single species trial

Single	Min	1st Q	Median	Mean	3rd Q	Max
Spyr19	0.000	0.000	1.500	3.333	6.000	10.000
Spyr24	0.000	2.500	4.000	4.143	6.000	8.000
Spyr29	1.000	2.500	5.000	4.667	6.750	8.000
Lgib19	4.000	8.000	9.000	8.111	9.000	10.000
Lgib24	1.000	3.500	7.000	5.571	7.500	9.000
Lgib29	6.000	9.500	10.000	9.286	10.000	10.000
Afac19	0.000	4.250	8.500	6.625	9.250	10.000
Afac24	8.000	9.500	10.000	9.500	10.000	10.000
Afac29	8.000	9.750	10.000	9.625	10.000	10.000

Table S2.4 **Number of Prey Captured in paired species trials.** Descriptive statistics for variable Number of Preys Captured of each paired species trial

Paired		Min	1st Q	Median	Mean	3rd Q	Max
SpyrSpyr19	**Spyr**	3.000	4.000	4.500	5.000	6.500	7.000
	Spyr	1.000	3.250	4.500	4.167	5.000	7.000
SpyrSpyr24	**Spyr**	0.000	2.000	3.000	3.714	6.000	7.000
	Spyr	0.000	0.000	1.000	1.429	2.500	4.000
SpyrSpyr29	**Spyr**	3.000	3.000	4.000	4.286	5.500	6.000
	Spyr	0.000	3.500	4.000	4.571	7.000	7.000
SpyrLgib19	**Spyr**	0.000	1.500	3.000	2.667	3.000	6.000
	Lgib	4.000	6.250	7.000	7.167	8.500	10.000
SpyrLgib24	**Spyr**	0.000	1.000	3.000	2.857	3.500	8.000
	Lgib	1.000	3.000	7.000	5.429	7.500	9.000
SpyrLgib29	**Spyr**	0.000	0.000	1.000	1.857	3.500	5.000
	Lgib	5.000	6.500	9.000	8.000	9.500	10.0
SpyrAfac19	**Spyr**	0.000	0.000	0.500	1.667	2.500	6.000
	Afac	3.000	4.750	8.000	7.167	9.750	10.000
SpyrAfac24	**Spyr**	0.000	0.000	0.000	0.000	0.000	0.000
	Afac	10.000	10.000	10.000	10.000	10.000	10.000
SpyrAfac29	**Spyr**	0.0000	0.0000	1.0000	0.5714	1.0000	1.0000
	Afac	6.000	9.000	9.000	9.000	10.000	10.000

Table S2.5 **Mean Time to Capture Preys in single species trials.** Descriptive statistics for variable Mean Time to Capture Prey, in seconds, of each single species trial

Single	Min	1st Q	Median	Mean	3rd Q	Max
Spyr19	5.000	11.250	17.500	14.830	19.750	22.000
Spyr24	4.000	11.000	27.750	38.080	57.620	96.000
Spyr29	2.000	3.875	13.500	15.420	22.000	38.000
Lgib19	2.000	2.000	2.500	3.222	4.000	6.500
Lgib24	1.000	1.750	2.000	4.429	7.500	9.500
Lgib29	1.000	1.000	1.000	1.571	2.000	3.000
Afac19	2.000	3.000	5.000	5.786	8.500	10.500
Afac24	1.000	1.000	1.250	1.438	2.000	2.000
Afac29	1.000	1.000	1.000	1.312	1.250	2.500

Table S2.6 **Mean Time to Capture Preys in paired species trials.** Descriptive statistics for variable Mean Time to Capture Prey, in seconds, of each paired species trial

Paired		Min	1st Q	Median	Mean	3rd Q	Max
SpyrSpyr19	**Spyr**	2.000	2.750	5.000	9.167	6.125	34.500
	Spyr	2.000	2.375	4.250	4.583	6.500	8.000
SpyrSpyr24	**Spyr**	4.000	11.500	20.500	23.830	31.000	55.000
	Spyr	6.500	14.750	22.750	32.000	40.000	76.000
SpyrSpyr29	**Spyr**	1.000	3.500	6.000	5.786	8.000	10.500
	Spyr	2.000	2.250	3.750	4.417	5.625	9.000
SpyrLgib19	**Spyr**	1.000	1.500	2.000	2.300	3.000	4.000
	Lgib	1.000	1.125	1.500	1.500	1.875	2.000
SpyrLgib24	**Spyr**	0.000	1.250	3.000	3.667	5.500	9.000
	Lgib	0.000	1.500	3.000	3.786	3.500	13.500
SpyrLgib29	**Spyr**	1.000	1.375	2.250	2.125	3.000	3.000
	Lgib	1.000	1.000	1.000	1.286	1.500	2.000
SpyrAfac19	**Spyr**	2.000	2.750	3.500	31.170	45.750	88.000
	Afac	2.000	2.000	2.000	2.500	2.750	4.000
SpyrAfac24	**Spyr**	-	-	-	-	-	-
	Afac	1.000	1.750	2.000	2.286	2.500	4.500
SpyrAfac29	**Spyr**	2.000	2.750	15.000	27.250	39.500	77.000
	Afac	1.000	1.000	1.000	1.000	1.000	1.000

APPENDIX III

Table S3.1 **Sampling information.** Number, mean total length and range (mm) of fish caught and used in experiments, and mean water temperature (ºC), on each sampling date and location

Date	*S. pyrenaicus* (S. Martinho)			*L. gibbosus* (Corona)			*A. facetus* (Corona)			Mean Water Temperature	
	N	Mean TL	Range	N	Mean TL	Range	N	Mean TL	Range	S. Martinho	Corona
Jan 2016	2	55.5	50-61	11	59.4	48-74	10	59.2	49-73	16.6	13.9
Mar 2016	17	63.7	45-78	5	66.6	53-75	11	55.6	48-66	14.0	12.4
May 2016	25	60.3	48-73	9	62.3	53-70	8	60.6	48-70	16.4	-
Aug 2016	17	67.5	59-76	3	65.7	65-66	0	-	-	21.4	24.2
Oct 2016	19	65.1	57-76	9	63.1	53-76	9	67.3	58-75	14.8	19.2
Dec 2016	13	67.9	61-80	4	67.0	64-72	3	66.0	61-70	9.7	10.0
Apr 2017	4	62.0	49-77	2	53.5	51-56	3	65.0	53-74	14.1	17.4
May 2017	2	52.5	49-56	0	-	-	0	-	-	17.4	14.1
Total	99	62.7	45-80	43	62.5	48-76	44	62.3	48-75	15.6	15.9